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FULL PAPER

Synthesis of Morpholine-Based Analogs of (−)-Zampanolide and Their Biological Activity

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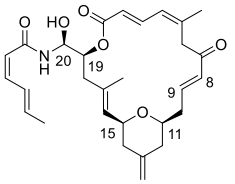
Abstract: We describe the convergent synthesis of three prototypical examples of a new class of analogs of the complex, cytotoxic marine macrolide (−)-zampanolide, which incorporate an embedded *N*-substituted morpholine moiety in place of the natural tetrahydropyran ring. The final construction of the macrolactone core was based on a high-yielding intramolecular HWE olefination, while the hemiaminal-linked side chain was elaborated through a stereoselective, BINAL-H-mediated addition of (*Z*,*E*)-sorbamide to a macrocyclic aldehyde precursor. The synthesis of the common functionalized morpholine building block involved two consecutive epoxide openings with tosylamide and the product of the first opening reaction, respectively, as nucleophiles. Of the three morpholino-zampanolides investigated, the *N*-acetyl and the *N*-benzoyl derivative both exhibited nanomolar antiproliferative activity, thus being essentially equipotent with the natural product. In contrast, the activity of the *N*-tosyl derivative was significantly reduced.

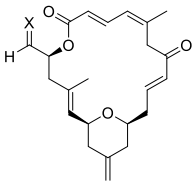
Introduction

Natural products are a highly prolific source of lead structures for drug discovery and development, owing to their coverage of structural space that is only sparsely populated by common synthetic organic molecules.[1] Thus, a substantial fraction of currently employed prescription drugs worldwide are either natural products themselves or can be considered as being natural product-derived.[2] Historically, the vast majority of bioactive natural products have been obtained from terrestrial plants, bacteria, or fungi;[3] however, more recently, natural products originating from marine organisms, such as sponges, corals, algae, or marine bacteria have attracted significant attention as potential leads for drug discovery[4] or as probes in chemical biology.[5] While these compounds are generally more difficult to access than terrestrial natural products and often can only be obtained in very small quantities, marine natural products have been argued to show a higher incidence of significant bioactivity, which is often associated with a high degree of structural novelty.[6] At this point in time, 13 marine natural products have been developed into FDA-approved drugs (including 3 antibody-drug conjugates), while 23 compounds are undergoing Phase I, II or III clinical trials (including 15 antibody-drug conjugates).[4a],[7] While these numbers may appear relatively small, they have to be judged in relation to the total number of marine natural products currently known (around 30'000),[4c] which is much smaller than the number of terrestrial natural products (>300'000).[8]

(−)-Zampanolide (**1**) is a macrolide that was first isolated in 1996 by Tanaka and Higa from the marine sponge *Fasciospongia rimosa*.[9] The compound was found to inhibit the proliferation of several cancer cell lines in vitro with nanomolar potency, although the underlying mechanism of action was not investigated.



(−)-Zampanolide (**1**)



2: X = O
3: X = H, OH

In 2009, (−)-zampanolide (**1**) was re-isolated from the Togan sponge *Cacospongia mycofijensis* by Field *et al*.[10] who also demonstrated that **1** was a potent microtubule-stabilizing agent

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(MSA) which arrests cells in the G2/M phase of the cell cycle and induces apoptosis.[10] Thus, (−)-zampanolide (**1**) inhibits cancer cell growth through the same mechanism as the established anticancer drugs paclitaxel, taxotere, cabazitaxel, and ixabepilone. [11]

As first demonstrated by biochemical experiments, **1** binds to the taxol-binding site on β-tubulin in a covalent fashion.[12] These conclusions were subsequently confirmed by the high resolution X-ray crystal structure of the tubulin/zampanolide complex;[13] the structure also revealed that the covalent attachment of **1** to β-tubulin is based on the 1,4-addition of the imidazole moiety of βHis229 to the enone moiety in the macrocycle.

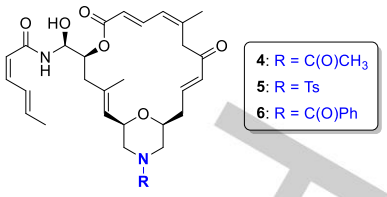
Structurally, (−)-zampanolide (**1**) is characterized by a highly unsaturated 20-membered macrolactone core with an embedded *syn*-2,6-disubstituted tetrahydropyran ring carrying an exocyclic methylene group. Attached to C(19) of this macrolactone core is a (*Z,E*)-sorbamide-containing side chain that also includes a highly unusual hemiaminal group which connects the sorbamide moiety to the macrocycle. Only few other natural products are known that incorporate related *N*-acyl hemiaminal groups.[14]

Given its intriguing structural features and its medically relevant mode of action, it is unsurprising that (−)-zampanolide (**1**) has been the target of several total syntheses,[15] including work from our own group.[15e] Prior to these efforts, Smith had reported the synthesis of *ent*-**1**;[16] this work established the absolute configuration of the natural product to be 11*S*, 15*S*, 19*S*, 20*S*.

Work has also been reported on the synthesis and biological evaluation of analogs of **1**,[15e],[17] but overall, the SAR around the compound is still underexplored. Importantly, however, it has been demonstrated that the C(19) side chain is crucial for the potent biological activity of **1**, as both aldehyde **2** and alcohol **3** are significantly less potent than **1**.[15e],[18] On the other hand, we have shown that the removal of the exocyclic methylene group from either **1**[19] or **2/3**[15e],[18b] has no significant effect on biological potency. This observation is in line with the structural data on the zampanolide/β-tubulin complex, which show no significant interactions between the methylene group and the protein.[13]

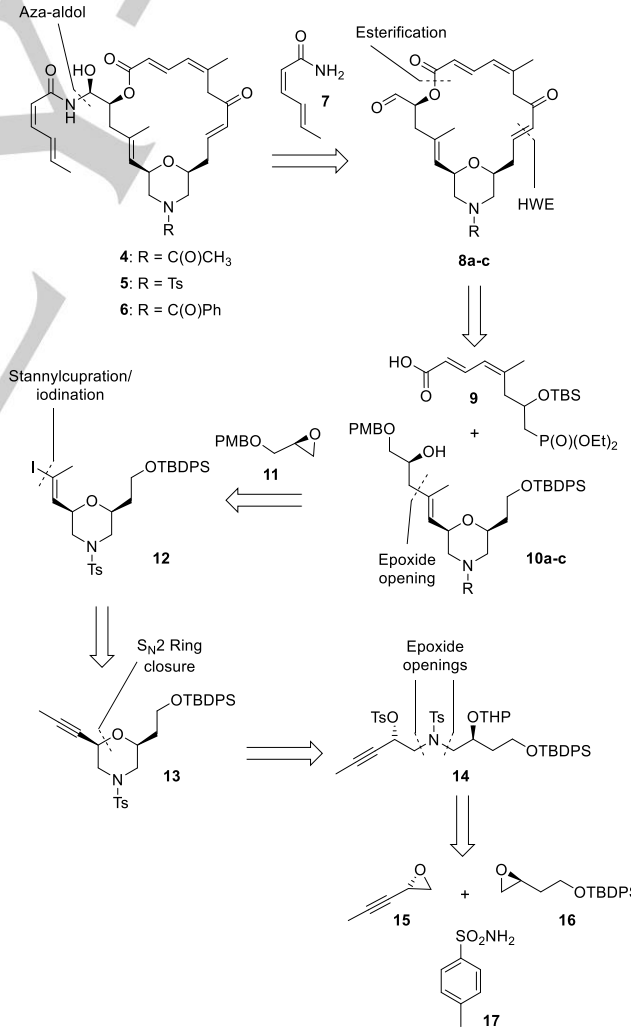
Based on our findings for the 13-desmethylene derivatives of **1**, **2**, and **3**, we became interested in analogs of **1**, where the THP ring would be replaced by a morpholine moiety. We felt that the morpholine *N*-atom could serve as a convenient site for the attachment of a variety of substituent groups to modulate the physico-chemical and biological properties of such analogs, including the attachment of tumor-targeting and/or prodrug moieties. In light of these considerations, it was the objective of this work to establish an efficient platform for the synthesis of *N*-substituted morpholino-zampanolides and to collect some preliminary biological data for a select number of derivatives. As we felt it important to understand the size tolerance for substituents on the morpholine nitrogen, acetamide **4** and sulfonamide **5** were chosen as initial target structures for synthesis and biological evaluation; based on the biological results with **4** and **5**, we subsequently extended our synthetic work to benzamide **6**.

In the following, we describe the successful synthesis of morpholino-zampanolides **4**-**6** and the assessment of their microtubule-binding affinity and antiproliferative activity.



Results and Discussion

Synthetic planning. Our overall strategy for the synthesis of morpholino-zampanolides **4**-**6** was to build on our previous work on the total synthesis of (−)-zampanolide (**1**)[15e] and of zampanolide analogs[19] to the largest extent possible; including the usage of building blocks that had served as intermediates in the synthesis of **1**. Thus, the C(19) side chain was to be elaborated in the last step of the synthesis by an aza-aldol reaction of aldehydes **8a-c** with (*Z,E*)-sorbamide (**7**) (Scheme 1), employing methodology that has recently been developed in our laboratory for the stereoselective establishment of the hemiaminal center in zampanolide-type structures.[19]



Scheme 1. Retrosynthesis of morpholino-zampanolides **4**, **5**, and **6**. **a**: R = $C(O)CH_3$; **b**: R = Ts; **c**: R = C(O)Ph.

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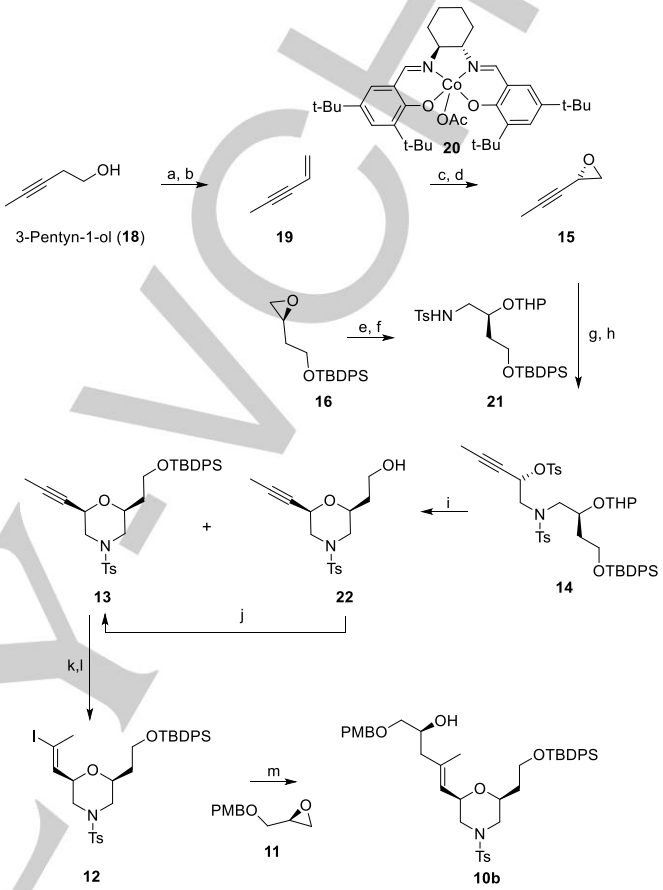
The macrocycle was envisioned to be closed by an intramolecular Horner–Wadsworth–Emmons (HWE) reaction; the macrocyclization precursors for the HWE reaction would be obtained by esterification of the acid **9**[15e] with morpholine derivatives **10a-c**. The latter were to be accessed from vinyl iodide **12** via iodine/metal exchange and subsequent reaction of the vinylmetal species with PMB-protected (*R*)-glycidol (**11**). For amide-based target structures **4** and **6**, this was to be followed by cleavage of the tosyl group and acylation. Vinyl iodide **12** would be derived from alkyne **13** by a stannylcupration/Sn-iodine exchange sequence. The construction of the crucial morpholino core with the appropriately functionalized appendices at positions 2 and 6 (morpholine numbering) was considered one of the major challenges in the synthesis of **4**-**6**, given the scarcity of methods that are available for the synthesis of systems similar to **13**. We opted to approach the problem based on methodology that has been developed by Penso and co-workers for the stereoselective preparation of 2,6-disubstituted *N*-tosyl morpholines.[20] The method entails opening of a first epoxide with tosyl amide (**17**), protection of the resulting β-hydroxyethyl sulfonamide as a THP-ether, base-catalyzed opening of a second epoxide with the sulfonamide as a nucleophile, tosylation of the ensuing free hydroxy group, cleavage of the THP-ether, and final base-mediated ring-closure to the morpholine. Based on this protocol, morpholine derivative **13** was envisioned to be accessible from tosyl amide (**17**) and the known epoxides **15**[21] and **16**[15e] *via* sulfonamide **14**. The latter would be obtained if the first step of the above reaction sequence involved opening of **16** with **17**. While this approach inevitably leads to *N*-tosyl morpholines, we felt confident that the removal of the tosyl group at the appropriate stage would be feasible.

Synthesis of alcohol 10b. The synthesis of building block **10b** in a first stage required the preparation of the known epoxides **15**[21] and **16**[15e] (Scheme 2). The synthesis of **15** involved the tosylation of commercially available 3-pentyn-1-ol (**18**), followed by base-induced elimination to furnish pent-2-en-4-yne (**19**) in 84% overall yield.[22] Ene-yne **19** was then converted into the enantiopure epoxide **15** by a Jacobsen epoxidation/(salen)cobalt-catalyzed hydrolytic kinetic resolution (HKR) sequence in 39% yield.[21]

Epoxide **16** was obtained in 3 steps from D-aspartic acid according to Zurwerra *et al*.[15e] Ring-opening of **16** with *p*-toluenesulfonamide (*p*-TsNH$_2$; **17**) under solid-liquid phase-transfer catalysis (SL-PTC) conditions[20] and subsequent protection of the resulting secondary alcohol as a THP-ether then gave the fully protected aminodiol **21** as a mixture of diastereomers (at C(2) of the THP ring). Reaction of tosyl amide **21** with oxirane **15** under the same phase transfer conditions employed for the ring-opening of **17** with *p*-TsNH$_2$,[20] followed by tosylation of the ensuing secondary hydroxy group by reaction with TsCl in DCM furnished tosylate **14** in 54% overall yield (from **21**). Initial attempts at the removal of the THP protecting group under the conditions reported by Penso, i. e. treatment of **14** with pyridinium *p*-toluenesulfonate (PPTS) in aqueous ethanol at 55 °C,[20] only led to decomposition. Gratifyingly, this problem could be overcome by conducting the reaction at room temperature; in contrast to the examples reported by Penso *et al*., these conditions not only led to cleavage of the THP-ether but directly gave the ultimately desired morpholine derivative **13** in 48% isolated yield. In addition, the free alcohol **22** was obtained in 24% yield, resulting from partial cleavage of the TBDPS-ether group, either before or after morpholine ring formation. Alcohol **22** could be readily reprotected under standard conditions (TBDPSCl, imidazole, DMAP in DMF) to furnish **13** in 92% yield, thus bringing the total yield of **13** to 70% (from **14**). The route depicted in Scheme 3 was highly scalable and provided **13** in multigram quantities.



Scheme 2. a) *p*-Toluenesulfonyl chloride, NEt$_3$, dichloromethane, 0 °C → rt, 24 h, 97%; b) KOH, Teepol™, H$_2$O, 160 °C, 4 h, 86%; c) NaOCl, (*R*,*R*)-Jacobsen's catalyst, dichloromethane, 0 °C, 15 h, 62%, *ee*, 48%; d) (*S*,*S*)-Co-salen complex (**20**), H$_2$O, Et$_2$O, 0 °C → rt, 22 h, 65%, ee ≥ 99.5%; e) *p*-toluenesulfonamide (**17**), benzyltriethylammonium chloride, K$_2$CO$_3$, dioxane, 90 °C, 24 h, 81%; f) 3,4-dihydropyran, pyridinium *p*-toluenesulfonate, dichloromethane, rt, 1.5 h, quant.; g) **21**, benzyltriethylammonium chloride, K$_2$CO$_3$, dioxane, 90 °C, 20 h, 58%; h) *p*-toluenesulfonyl chloride, NEt$_3$, 4-(dimethylamino)pyridine, dichloromethane, 0 °C → rt, 3.5 h, 93%; i) pyridinium *p*-toluenesulfonate, aq. EtOH, rt, 40 h, 48% (**13**), 24% (**22**); j) *tert*-butyldiphenylchlorosilane, imidazole, 4-(dimethylamino)pyridine, *N,N*-dimethylformamide, rt, 2 h, 92%; k) CuCN, *n*-BuLi, Bu$_3$SnH, MeOH, tetrahydrofuran, −78 °C → −15 °C, 16.5 h, 96%; l) *N*-iodosuccinimide, tetrahydrofuran, −18 °C, 75 min, 74% (94%; see text); l) *n*-BuLi, BF$_3$•OEt$_2$, toluene, −78 °C → −100 °C, 2 h, 76%.

With a scalable route to morpholine derivative **13** established, the alkyne functionality was then converted into the corresponding vinyl iodide moiety by a stannylcupration/iodination sequence with Bu$_3$Sn(Bu)CuCNLi$_2$[23] and *N*-iodosuccinimide (NIS) (Scheme 2). The stannylated product was obtained in excellent yield (96%) as a single isomer. Vinyl iodide **12** was isolated in 74% yield after column chromatography. In addition to **12**, significant amounts of the stannane precursor were recovered, although TLC analysis had indicated complete conversion. The recovered stannane was re-subjected to the iodination conditions, but in contrast to the previous experiment, the excess NIS was not quenched with

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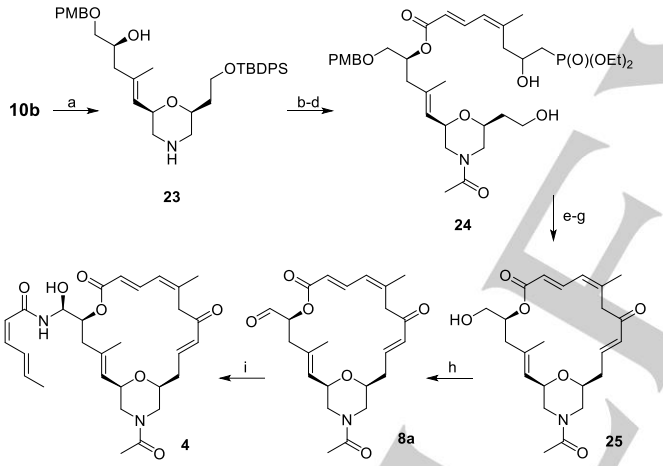
aqueous sodium thiosulfate. Instead, the reaction mixture was diluted with hexanes and directly purified by column chromatography. In doing so, the desired product **12** could be isolated in 74% yield. (Resubmitting the recovered intermediate stannane to reaction with *N*-iodosuccinimide gave additional **12**, brining the total isolated yield to 94%). The subsequent iodine-lithium exchange followed by reaction of the ensuing vinylmetal species with PMB-protected (*R*)-glycidol (**11**) afforded alcohol **10b** in 76% yield.

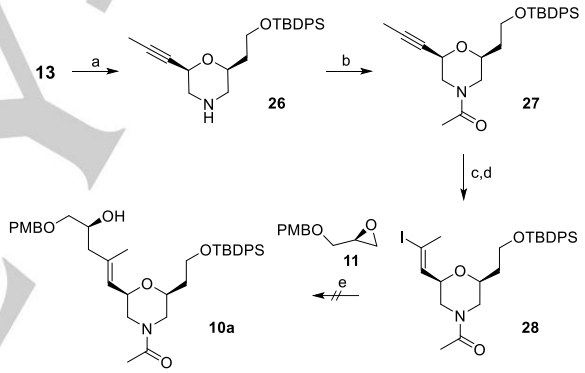
Synthesis of morpholino-zampanolide 4. The elaboration of alcohol **10b** into the *N*-acetyl morpholine-based zampanolide analog **4** in the first step involved cleavage of the tosyl group from the morpholine nitrogen with magnesium turnings in methanol,[24] to afford the free amine **23** in 85% yield (Scheme 3). The latter could then be acetylated in almost quantitative yield with isopropenyl acetate;[25] subsequent esterification of this intermediate with acid **9** under Yamaguchi conditions[15e],[26] followed by global desilylation afforded diol **24** in 84% yield over 2 steps. Oxidation of **24** with Dess-Martin periodinane[27] gave a keto aldehyde that underwent smooth $Ba(OH)_2$-mediated HWE reaction,[15e],[28] to furnish the desired macrolactone in 68% yield (based on **24**). Oxidative PMB-cleavage with DDQ then provided primary alcohol **25**, which was readily oxidized to aldehyde **8a** with Dess-Martin periodinane.[27]



Scheme 3. a) Mg, MeOH, 0 °C → rt, 2.5 h, 85%; b) isopropenylacetate, 60 °C, 3 h, 96%; c) **9**, 2,4,6-trichlorobenzoyl chloride, NEt_3, 4-(dimethylamino)pyridine, tetrahydrofuran, rt, 2.5 h, quant.; d) HF•pyridine, tetrahydrofuran, 0 °C → rt, 14 h, 84%; e) Dess-Martin periodinane, dichloromethane, rt, 55 min; f) $Ba(OH)_2$, tetrahydrofuran / H_2O 40:1, 0 °C, 12 min, 68% over 2 steps; g) 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, dichloromethane / phosphate buffer (pH 7.2) 40:1, rt, 2 h, 98%; h) Dess-Martin periodinane, $NaHCO_3$, dichloromethane, rt, 50 min, 78%; i) **7**, $LiAlH_4$, EtOH, (*S*)-(−)-1,1'-bi(2-naphthol), tetrahydrofuran, rt, 18 min, 15%, dr > 99:1.

The addition of (*Z,E*)-sorbamide (**7**) to aldehyde **8a** was then performed using methodology that we have recently developed for the stereoselective establishment of the hemiaminal center in zampanolide-type structures.[19] Thus, **7** was added to a solution of (*S*)-BINAL-H[29] to form a putative amide transfer complex; this solution was then added immediately to a THF solution of **8a** and the mixture was stirred for 18 min. In contrast to other examples investigated,[19] the addition of only 2 equiv of the putative (*S*)-BINAL-**7** complex resulted in almost no conversion of **8a**. To achieve full conversion, 5 portions of 2 equiv. of (*S*)-BINAL-**7** complex solution were added over 10 min. The *N*-acetyl morpholine-based zampanolide analog **4** was obtained in 75% yield after flash column chromatography (FC) (according to ^{1}H-NMR of the FC-purified material); for reasons that have not been explored, the isolated yield after preparative NP-HPLC purification was only 15%. **4** was obtained with a dr of >99:1.[30]

In an alternative approach towards analog **4**, we have also explored the removal of the *N*-tosyl group at the stage of alkyne **13** (Scheme 4). Different desulfonation conditions were examined in this context (Na in pentanol[31], TMSCl and NaI in acetonitrile[32] and Mg in MeOH[24]), of which only magnesium in methanol delivered the desired product in a reasonable yield. The free morpholine **26** was acetylated with acetic anhydride to provide **27** in excellent yield. The latter was converted into vinyl iodide **28** by stannylcupration/iodination with $Bu_3Sn(Bu)CuCNLi_2$[23] and NIS. **28** was obtained in 88% yield; similar to the conversion of alkyne **13** into vinyl iodide **12** (cf. Scheme 2), 12% of the intermediate stannane was also recovered. Unfortunately, attempts at the elaboration of **28** into alcohol **10a** *via* iodine-lithium exchange were unsuccessful, using either *t*BuLi or *n*BuLi. Only complex product mixtures were obtained, possibly due to deprotonation of the acetyl residue.



Scheme 4. a) Mg, MeOH, 0 °C → rt, 19 h, 96%; b) acetic anhydride, pyridine, dichloromethane, -78 °C, 60 min, 91%; c) CuCN, *n*-BuLi, Bu_3SnH, MeOH, tetrahydrofuran, −78 °C → −15 °C, 21 h, 99%; d) *N*-iodosuccinimide, tetrahydrofuran, −17 °C → −10 °C, 30 min, 88% (+12% Sn-intermediate); e) *t*-BuLi or *n*-BuLi, BF_3•OEt_2, toluene, −78 °C → −100 °C, decomposition.

Synthesis of morpholino-zampanolide 5. The esterification of alcohol **10b** with acid **9**[15e] was again performed under Yamaguchi conditions[15e],[26] and the crude ester was directly submitted to silyl-ether cleavage with HF•pyridine, to afford diol **29** in 78% overall yield (Scheme 5). Double oxidation of **29** with DMP,[27] followed by a $Ba(OH)_2$-mediated macrocylization[15e] then provided macrolactone **30** in 69% overall yield (from **29**). Removal of the PMB-protecting group with DDQ followed by oxidation of the liberated hydroxy group with buffered DMP[27] gave aldehyde **8b** in 89% overall yield.

The elaboration of **8b** into morpholino-zampanolide analog **5** was again accomplished via the putative amide transfer reagent that is likely formed upon addition of (*Z,E*)-sorbamide (**7**) to a THF solution of (*S*)-BINAL-H.[19],[29] Again, 5 portions of 2 equiv. of the (*S*)-BINAL-**7** complex solution had to be added to aldehyde **8b** over 10 min, in order to achieve full conversion. None of the C(20)*R* epimer of **5** could be observed in the ^{1}H-NMR spectrum

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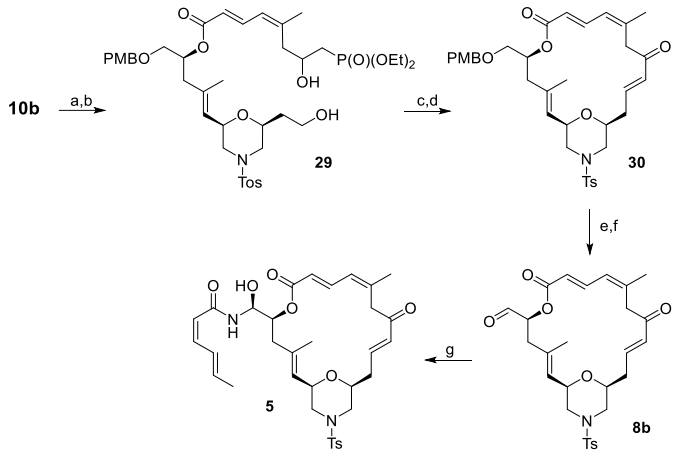
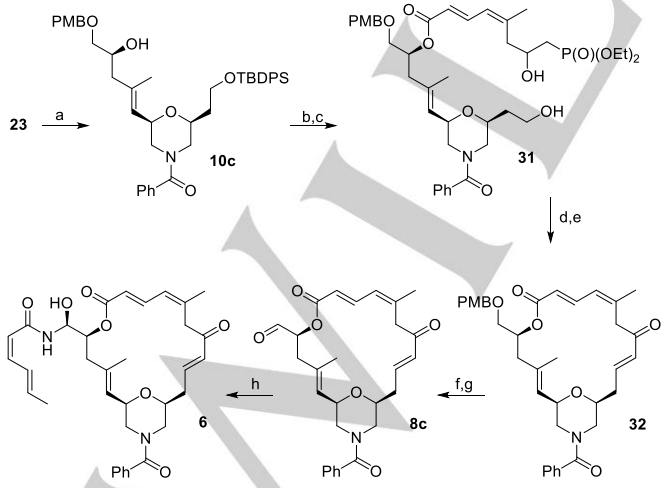
of the crude reaction mixture. After purification by flash column chromatography, **5** was obtained in 81% yield. The aza-aldol reaction between **8b** and **7** was also carried out with (*S)*-TRIP as a chiral Lewis acid catalyst, as described by Gosh *et al*. for the synthesis of (−)-zampanolide (**1**) from **2**.[15c],[15d] In line with the observations reported by Gosh, the reaction produced a ca. 2:1 diastereomeric mixture of **5** and its C(20)*R*-epimer in 68% yield (based on ^{1}H-NMR). The diastereomers were separable by preparative NP-HPLC.[30]



Scheme 5. a) **9**, 2,4,6-trichlorobenzoyl chloride, NEt_3, 4-(dimethylamino)pyridine, tetrahydrofuran, rt, 2 h; b) HF·pyridine, tetrahydrofuran, 0 °C → rt, 23 h, 78% over 2 steps; c) Dess-Martin periodinane, dichloromethane, rt, 25 min, d) $Ba(OH)_2$, tetrahydrofuran / H_2O 40:1, 0 °C, 15 min, 69% over 2 steps; e) 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, dichloromethane / phosphate buffer (pH 7.2) 5:1, rt, 74 min, quant.; f) Dess-Martin periodinane, $NaHCO_3$, dichloromethane, rt, 70 min, 89%; g) **7**, $LiAlH_4$, EtOH, (S)-(−)-1,1'-bi(2-naphthol), tetrahydrofuran, rt, 20 min, 81%, dr > 95:5.

Synthesis of morpholino-zampanolide 6. The synthesis of morpholino-zampanolide **6** from **23** followed the same sequence of transformations as described above for the synthesis of analog **4** (Scheme 6).



Scheme 6. a) PhC(O)Cl, NEt_3, dichloromethane, rt, 75 min, 98%; b) **9**, 2,4,6-trichlorobenzoyl chloride, NEt_3, 4-(dimethylamino)pyridine, tetrahydrofuran, rt, 3 h, 90%; c) HF·pyridine, tetrahydrofuran, 0 °C → rt, 21 h, 89%; d) Dess-Martin periodinane, dichloromethane, rt, 90 min; (e) $Ba(OH)_2$, tetrahydrofuran / H_2O 40:1, 0 °C, 14 min, 65% over 2 steps; (f) 2,3-dichloro-5,6-dicyano-*p*-benzoquinone, dichloromethane / phosphate buffer (pH 7.2) 40:1, rt, 90 min, 93%; (g) Dess-Martin periodinane, dichloromethane, rt, 90 min; h) **7**, $LiAlH_4$, EtOH, (S)-(−)-1,1'-bi(2-naphthol), tetrahydrofuran, rt, 20 min, 32% over 2 steps, dr > 99:1.

Thus, benzoylation of **23** with benzoyl chloride followed by Yamaguchi esterification of the ensuing benzamide **10c** with acid **9**[15e] and cleavage of both silyl-ethers with HF•pyridine furnished diol **31** in 78% overall yield. Double oxidation of **31** and $Ba(OH)_2$-mediated HWE ring-closure then gave the desired macrolactone **32** in 65% yield from **31**. While all other intermediates described in this study were oils, **32**, gratifyingly, proved to be crystalline; this allowed its skeletal structure and its absolute and relative configuration to be confirmed by X-ray crystallography (Fig. 1). This confirmation was particularly comforting as the presence of slowly interconverting rotamers around the tertiary amide bond led to extensive line broadening in the ^{1}H-NMR spectra of all intermediates as well as the final product **6**, to an extent that the signals for one proton of each of the CH_2 groups α to the amide nitrogen were virtually invisible; this was also the case for the corresponding ^{13}C-signals (see the SI for details). DDQ-mediated cleavage of the PMB group in **32**, DMP oxidation of the ensuing free hydroxy group, to give aldehyde **8c**, and (*S*)- BINAL-H-mediated aza-aldol reaction with (*Z,E*)-sorbamide (**7**) afforded the desired morpholino-zampanolide **6** in 30% overall yield after purification by preparative NP-HPLC.[30]

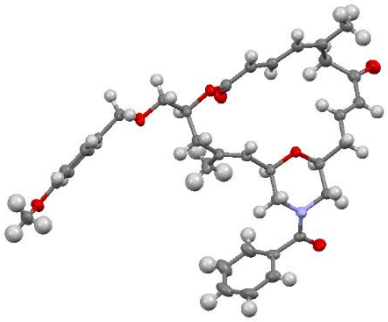


Figure 1. X-Ray structure of macrolactone **32**.

Biological assessment of morpholino-zampanolides 4-6. In order to establish a basic understanding of the biological consequences of the replacement of the methylenated tetrahydropyran ring in **1** by *N*-substituted morpholine moieties, the antiproliferative activity of morpholino-zampanolides **4-6** was assessed against different cancer cell lines in vitro. As can be seen from the data summarized in Table 1, both carboxamide derivatives **4** and **6** retain nanomolar antiproliferative potency and, thus, exhibit comparable activity as the natural product **1**.[33]
In comparison, and rather surprisingly, tosylamide **5** is ca. 2-3 orders of magnitude less potent than **4** or **6**. These activity differences are also evident at the level of the dactylolide analogs **8a** and **8b**, although to a lesser extent. The differences in antiproliferative activity between **4**, **5**, and **6** are in agreement with the differences in apparent microtubule-binding affinity as determined by the ability of the compounds to displace the fluroescent taxol derivative Flutax-2 from stabilized microtubules.[34] Thus, the apparent binding constants K_b at 35 °C

for **4** and **6** were determined as 6.16 x 10^7 M^{-1} and 0.80 x 10^7 M^{-1}, respectively (vs. 21.4 x 10^7 M^{-1} for **1**).[12] There is no strict correlation between microtubule-binding affinity and the antiproferative activity of microtubule-stabilzing agents[35] and these numbers are even less informative for covalent tubulin binders such as **1**; however, it is still significant that sulfonamide **5** was not able to displace taxol from microtubules even at a concentration of 25 μM, which is in line with its profoundly reduced growth inhibitory activity.

The synthesis of benzamide **6** was only embarked on after the cellular data for **4** and **5** had become available. The compound was meant to determine if the reduced potency of **5**, compared to **4**, was caused by the increased steric bulk of the nitrogen substituent or might be related to its different mode of attachment (sulfonamide vs. carboxamide). The data suggest that an amide linkage may indeed be a more preferred mode of attachment of substituent groups to the morpholine nitrogen. However, more examples need to be investigated, in order to confirm (or refute) this conclusion. Independent of this, and more important in the context of the current study, even our limited data set shows that the morpholino-zampanolide scaffold offers the potential for the development of new, highly potent antiproliferative agents.

Table 1. Antiproliferative activity of morpholino-zampanolides **4-6** and of (–)-dactylolide analogs **8a**, **8b** against human cancer cells. (IC_{50} values [nM]).[a]

Compound	A549 (lung)	PC-3 (prostate)	MC-7 (breast)	HeLa (cervical)
4	ND	1.7 ± 0.11	2.4 ± 0.13	1.3 ± 0.09
5	ND	1703 ± 258	1995 ± 205	1673 ± 155
6	6.7 ± 0.9	ND	ND	ND
1 [b]	3.2 ± 0.4	2.9 ± 0.4	6.5 ± 0.7	NA
8a	ND	229 ± 16	290 ± 43	189 ± 24
8b	ND	2440 ± 236	3095 ± 273	2990 ± 300
2 [b]	301 ± 4.3	751 ± 69	247 ± 2.6	NA

[a] Cells were exposed to compounds for 72 h. Protein content of fixed cells was then determined by methylene blue staining. For further experimental details, see ref. [36]. IC_{50} values are the mean of at least 3 independent experiments ± SD. ND = not determined; NA = not available from ref.[15e]. [b] Data from ref.[15e]

Conclusion

We have established an efficient route for the synthesis of morpholine-based analogs of the marine macrolide (−)-zampanolide (**1**), which is a potent microtubule-stabilizing agent and as such represents a potential lead for anticancer drug discovery. Building on the chemistry that we had developed in the context of our total synthesis of **1**, the final construction of the core macrolactone was based on macrocyclization by an intramolecular HWE reaction. Ring-closure proceeded very efficiently in all three cases investigated here. The successful transfer of this macrocyclization approach to a modified scaffold speaks to the robustness of the methodology (which has also been adopted by others).[17] The elaboration of the sorbamide-containing side chain relied on a new method recently developed in our laboratory for the stereoselective establishment of the hemiaminal center in zampanolide-type structures; the desired target structures were obtained in good yields and with high selectivity (even if the yield of **4** after NP-HPLC purification was only 15% (*vide supra*)). As a crucial element of our approach towards morpholino-zampanolides, the common morpholine building block **10b** could be accessed from epoxides **15** and **16** in 26% overall yield. Importantly, the removal of the tosyl group from the morpholine nitrogen in **10b** could be achieved without difficulty, thus offering the opportunity for the incorporation of a multitude of substituents late in the synthesis. While it would have been even more advantageous to deprotect the morpholine nitrogen only after macrocyclization, preliminary attempts to remove the tosyl group from macrolactone **30** were unsuccessful.

Although our sample size at this point is very small, the available data on cancer cell growth inhibition in vitro indicate that acyl substituents on the morpholine nitrogen in morpholino-zampanolides are well tolerated, even for the case of a relatively bulky benzoyl group. It will be important to determine if potent activity is still retained for even (much) larger groups, as this has important implications for the design of tumor-targeted conjugates, including antibody-drug conjugates with non-cleavable linkers. Somewhat surprisingly, sulfonamide **5** was substantially less potent than carboxamides **4** and **6**. It remains to be determined if the activity difference between **5** and **6** is caused by the *para*-methyl group in **5** (which is absent in **6**) or if it may result from the different mode of attachment of the (*para*-methyl) phenyl moiety to the morpholine nitrogen (sulfonamide vs. carboxamide).

In summary, we have established a reliable platform for the synthesis of *N*-substituted morpholino-zampanolides that should enable the synthesis of a broad variety of analogs of this type. The preliminary biological data obtained for **4** and **6** clearly suggest that a broader exploration of this new class of zampanolide congeners should be an interesting and promising endeavor. Work along these lines is ongoing in our laboratory.

Experimental Section

For all experimental details see the Supporting Information. Deposition Number 2026328 contains the supplementary crystallographic data for this paper.

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Keywords: macrocyclization • stereoselective aza aldol reaction • total synthesis • structure-activity relationship • zampanolide

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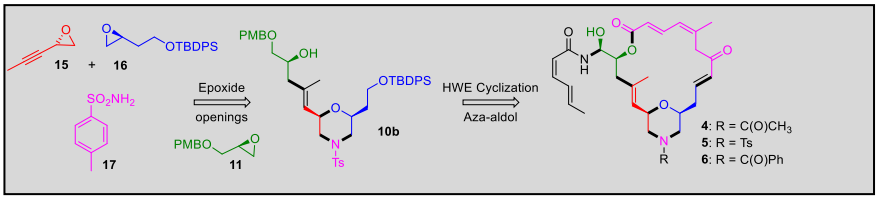

A synthetic approach has been developed towards a new class of analogs of the marine macrolide (–)-zampanolide, with a morpholine moiety replacing the tetrahydropyran ring. The morpholine core was elaborated from epoxides **15** and **16** and tosyl amide (**17**). The macrocycle was closed by a HWE reaction, and a stereoselective aza-aldol reaction installed the full sorbamide-derived side chain. Analogs **4** and **6** showed nM antiproliferative activity.

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